SOLID PHASE EXTRACTION OF BIOGENIC AMINES USING IMMOBILIZED NEW HYDRAZONE LIGANDS

by

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LIST OF ABBREVIATIONS

ACN	Acetonitrile
AGM	Agmatine
BA	Biogenic amines
C18	Octadecylsilane groups
CAD	Cadaverine
CE	Capillary electrophoresis
CHN	Carbon, hydrogen and nitrogen elemental analysis
DAD	Diode array detector
DAPCH	1-(3,4–dihydroxybenzaldehyde)–2–acetylpyridiniumchloride hydrazone
DMSO	Dimethylsulfoxide
Dn-Cl	Dansyl chloride
ELISA	Enzyme-linked immunosorbent assays
EtOH	Ethanol
FL	Fluorescence
FTIR	Fourier transform infrared spectroscopy
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
HBH	4-hydroxybenzoylhydrazine
HCl	Hydrochloric acid
HIS	Histamine
HL	Hydrazone ligand
HPLC	High performance liquid chromatography
LC	Liquid chromatography
LC-MS	Liquid chromatography-mass spectrometry
LIF	Laser induced fluorescence

LLE	Liquid-liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
LPME	Liquid phase microextraction
MeOH	Methanol
$NaHCO_3$	Sodium hydrogen carbonate
NaOH	Sodium hydroxide
NQS	1,2-naphthoquinone-4-sulfonatemethanol
NR	Not reported
OPA	*o*-phthalaldehyde
P.S.I	Pounds per square inch
PDA	Potato dextrose agar
PEA	β-phenylethylamine
PSE	1-pyrenebutanoic acid succinimidyl ester
PUT	Putrescine
PVC	Polyvinylchloride
r^2	Square of regression coefficient
RSD	Relative standard deviation
SD	Standard deviation
SPD	Spermidine
SPE	Solid phase extraction
SPM	Spermine
SPME	Solid phase microexteaction
TEOS	Tetraethoxyorthosilane
TGA	Thermogravimetric analysis
THF	Tetrahydrofuran
TLC	Thin layer chromatography

TRY	Tryptamine
TYR	Tyramine
UDLLME	Ultrasound-assisted depressive liquid-liquid microextraction
UV-Vis	Ultraviolet and visible
Wt	Weight
^{1}H NMR	Proton nuclear magnetic resonance spectroscopy

PENGEKSTRAKAN FASA PEPEJAL TERHADAP AMINA BIOGENIK MENGGUNAKAN LIGAN HIDRAZON BARU TERPEGUN

ABSTRAK

Dalam kajian ini, sebatian hidrazin dan hidrazid melalui tindak balas kondensasi dengan keton dan terbitan berbeza aldehid menghasilkan hidrazon sepadan yang dijangkakan. Kesemua ligan yang dipisahkan memperolehi hasil yang memuaskan (82-94 %). Lapan ligan hidrazon iaitu benzofenon-2,4-dinitrofenilhidrazon (HL1), formaldehid-2,4-dinitrofenilhidrazon (HL2), salisilaldehid-2,4-dinitrofenilhidrazon (HL3), 3-hidroksi-4-nitrobenzaldehid-2,4-dinitrofenilhidrazon (HL4), 3-hidroksi-4-nitrobenzaldehid-4-hidroksibenzoilhidrazone (HL5), 2-hidroksi-5-nitrobenzaldehid-2,4-dinitrofenilhidrazon (HL6), 2-hidroksi-5-nitrobenzaldehid-4-hidroksibenzoilhidrazon (HL7) dan salisilaldehid-4- hidroksibenzoilhidrazon (HL8) telah disintesis dan dicirikan. Struktur kimia ligan-ligan ini adalah konsisten dengan data analisis dan spektroskopi (CHN, FTIR, UV-Vis, TGA dan ^{1}H NMR). Ligan-ligan yang diperolehi diuji untuk aktiviti anti-mikrobial terhadap fungi; *Aspergillus niger, Aspergillus flavus dan Aspergillus fumigitus* dan bacteria; *Staphylooccus sp* dan *Enterobakteria sp*. Keputusan menunjukkan kesemua sebatian ini muncul sebagai agen antibakteria berbanding antifungal.

Lapan bahan penjerap (Sol1-Sol8) berasaskan kepada ligan hidrazon yang disintesis (HL1-HL8), telah disediakan secara pemegunan ligan-ligan tersebut ke atas matrik silica sol-gel. Keupayaan bahan penjerap untuk mengekstrak tujuh amina biogenik (BA) iaitu triptamina (TRY), β-feniletilamina (PEA), putrescina (PUT), cadaverina (CAD), histamina (HIS), tiramina (TYR) dan spermidina (SPD) telah dikaji

menggunakan kaedah serapan kelompok. Ekstrak dianalisis menggunakan kromatografi cecair prestasi tinggi (HPLC) dan pengesan ultralembayung (254 nm). Keputusan yang diperolehi menunjukkan penjeraap Sol1 berasaskan hidrazon (HL1) pada pH 9 menunjukkan kepilihan yang lebih terhadap amina alifatik (PUT, CAD, SPD) dan heterosiklik (HIS) (%E > 96) berbanding aromatic (TRY, PEA, TYR) (%E 78.7, 46.4 dan 82.0 masing-masing). Keupayaan pengekstrakan bahan penjerap ini yang tinggi diperolehi disebabkan oleh afiniti di antara penjerap sol-gel yang mengandungi ligan hidrazin dan analit yang dikehendaki. Bahan penjerap ini boleh digunakan sehingga enam kali pengekstrakan terhadap SPD, CAD dan PUT dan telah digunakan untuk penentuan BA di dalam sampel makanan yang berbeza (sos, jus oren dan kicap) yang telah ditambahkan dengan 5 mg L^{-1} BA piawai. Analit yang telah diekstrak diterbitkan menggunakan dansil klorida sebelum penentuan HPLC. Perolehan semula yang memuaskan (84.1 - 106.7%) telah diperolehi untuk kesemua analit dalam sampel makanan yang telah diuji.

ABSTRACT

In this work, hydrazine and hydrazide compounds were subjected to condensation reaction with ketone and different aldehyde derivatives affording the corresponding expected hydrazones. All the ligands were isolated in satisfactory yields (82 – 94%). Eight hydrazone ligands, namely, benzophenone - 2,4 - dinitrophenylhydrazone (HL1), formaldehyde-2,4–dinitrophenylhydrazone (HL2), salicylaldehyde–2,4– dinitrophenyl-hydrazone (HL3), 3-hydroxy-4-nitrobenzaldehyde-2,4-dinitrophenylhydrazone (HL4), 3-hydroxy-4-nitrobenzaldehyde-4-hydroxybenzoylhydrazone (HL5), 2-hydroxy-5-nitro-benzaldehyde-2,4-dinitrophenylhydrazone (HL6), 2-hydroxy-5-nitrobenzaldehyde-4-hydroxybenzoylhydrazone (HL7) and salicylaldehyde-4-hydroxybenzoylhydrazone (HL8) have been synthesized and characterized. Their chemical structures were found to be consistent with both analytical and spectroscopic data (CHN, FTIR, UV/Vis, TGA and ^{1}H NMR). The obtained ligands were evaluated for their anti-microbial activity towards fungi, *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus* and bacteria, *Staphylooccus* sp and *Enterobacter* sp. The results showed that these compounds are mostly antibacterial rather than antifungal agents.

Eight sorbent materials (Sol1-Sol8) based on the newly synthesized hydrazone ligand (HL1-HL8), were prepared by immobilizing the ligands into a silica sol-gel matrix. The capability of the sorbent materials for the extraction of seven biogenic amines (BA), i.e., tryptamine (TRY), *β*-phenylethylamine (PEA), putrescine (PUT), cadaverine (CAD), histamine (HIS), tyramine (TYR), and spermidine (SPD) was studied by the batch sorption method. The extracts were analysed by high-performance liquid chromatography (HPLC) with ultraviolet detection (254 nm). Results obtained showed that sorbent Sol1 based hydrazone (HL1) at pH 9, exhibited preferential selectivity towards the aliphatic

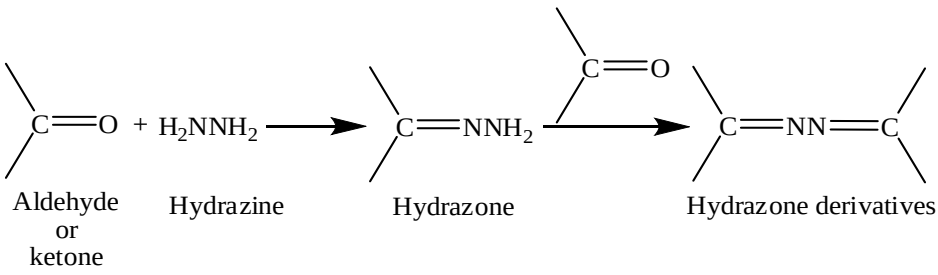
(PUT, CAD, SPD) and the heterocyclic (HIS) (%E > 96) over the aromatic (TRY, PEA, TYR) BA (%E 78.7, 46.4 and 82.0 respectively). The high extraction efficiency of the sorbent was achieved due to the high affinity between the sol-gel sorbent that contained the hydrazone ligand and the target analyte. The sorbent could be used up to six extraction cycles for SPD, CAD and PUT and was applied to the determination of BA in different food samples (ketchup, orange juice, soy sauce) that were spiked with 5 mg L^{-1} of the standard BA. The extracted analytes were derivatized with dansyl chloride before the HPLC determination. Reasonable recoveries (84.1 - 106.7%) were found for all analytes in the tested food samples.

CHAPTER ONE

INTRODUCTION

1.1 Hydrazones

Hydrazones are organic compounds of the formula $R_1C{=}N{-}NR_2$ where R_1 and R_2 represent H, aliphatic and aromatic group. They are usually formed by the condensation reaction between hydrazine and ketones or aldehydes. The oxygen atoms in aldehydes and ketones are replaced with the $N{-}NR_2$ functional group. Hydrazones are practically insoluble in hot and cold water but some are partially soluble in cold ethyl alcohol and ether. They possess a free amino group and can condense with another molecule of the carbonyl component to form hydrazone derivative or azine (Fieser and Fieser, 1956). The hydrazone unit offers a number of attractive features: a degree of rigidity, a conjugate π-system and an NH unit that can readily participate in hydrogen bonding and may be a site of protonation and deprotonation (Beves *et al.,* 2009). The formation of hydrazone and its derivatives can be presented by the following equation:

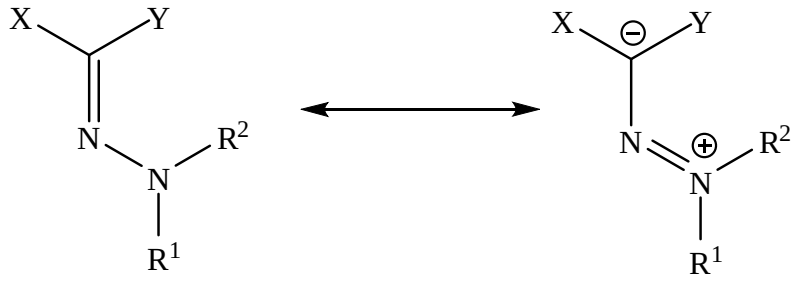


(1.1)

Derivatives of hydrazine in which this double condensation is obviated by the presence of a substituent group have more practical value than hydrazine. One of them is phenylhydrazone that are formed from phenylhydrazine and benzaldehyde. Replacement of the oxygen atom of the carbonyl group by $=NNHC_6H_5$ is followed by the increase in molecular weight and decrease in solubility. An aldehyde or a ketone can often be precipitated from a dilute solution as phenylhydrazone and identified by the melting point of this derivative (Fieser and Fieser, 1956).

Accordingly, various hydrazone derivatives with different applications and properties were prepared by the reaction of hydrazine with different aldehydes and ketones. Hydrazone derivatives have been demonstrated to possess antimicrobial, anticonvulsant, antidepressant, analgesic, anti-inflammatory, anti-platelet, anti-tubercular, anti-tumoral activities, etc.

Hydrazones are organic compounds in which the carbon functional group exist in the X and Y positions as CO_2R, CN are usefull in dye industry (Hunger *et al*., 2003). Nitrogen lone pair resonance (Scheme 1.1) made the hydrazone to have the carbon atom electron rich and the nucleophilicity of this carbon atom has been used extensively in the synthesis (Hunger *et al*., 2003). Due to their theoretical and practical importance, the structures and synthetsis of these molecules have been extensively studied. In this part of our study we review the chemistry of this class of compounds.



Scheme 1.1: Nitrogen lone pair resonance (R_1 and R_2 = H, aliphatic or aromatic group)

1.2 Hydrazines

Hydrazine compounds or derivatives are the main starting material in the synthesis and preparation of hydrazone derivatives. Hydrazine, having the formula $H_2N–NH_2$ (N_2H_4), can be considered as a nitrogen analog of hydrogen peroxide. It has an ammonia-like odour and is derived from ammonia but its physical properties are similar to water. It is a weak base which is protonated to give the $N_2H_5^+$ ions according to equation (1.2):

$$N_2H_4\,(aq) + H_2O\,(l) \rightleftharpoons N_2H_5^+\,(aq) + OH^-\,(aq) \qquad \textbf{(1.2)}$$

Hydrazine has an endothermic enthalpy of formation; therefore it is an unstable compound (Brady, 1990). It is a powerful reducing agent and reacts vigorously with strong oxidizing agents such as the halogens, nitric acid, dinitrogen tetraoxide and hydrogen peroxide to give large volume of gaseous products according to equations (1.3) and (1.4). It has been used as rocket fuels because the combustion of hydrazine is very exothermic (Gillepie *et al.*, 1989).

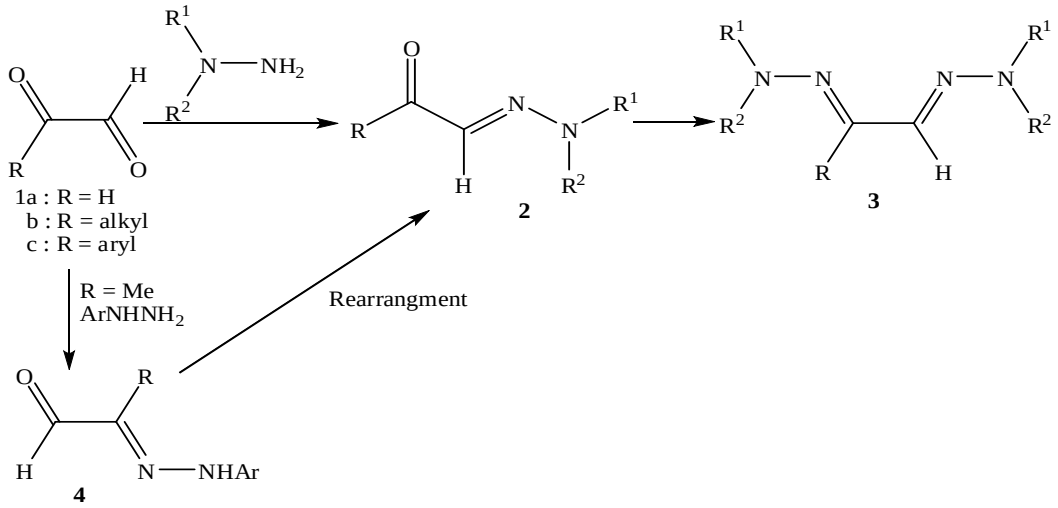
$$2\,N_2H_4\,(l) + N_2O_4\,(l) \longrightarrow 3\,N_2\,(g) + 4\,H_2O\,(g) \qquad \textbf{(1.3)}$$

$$N_2H_4\,(l) + 2\,H_2O_2\,(l) \longrightarrow N_2\,(g) + 4\,H_2O\,(g) \qquad \textbf{(1.4)}$$

1.3 Synthesis of hydrazones

1.3.1 Condensation of functionalized aldehydes and ketone with hydrazines

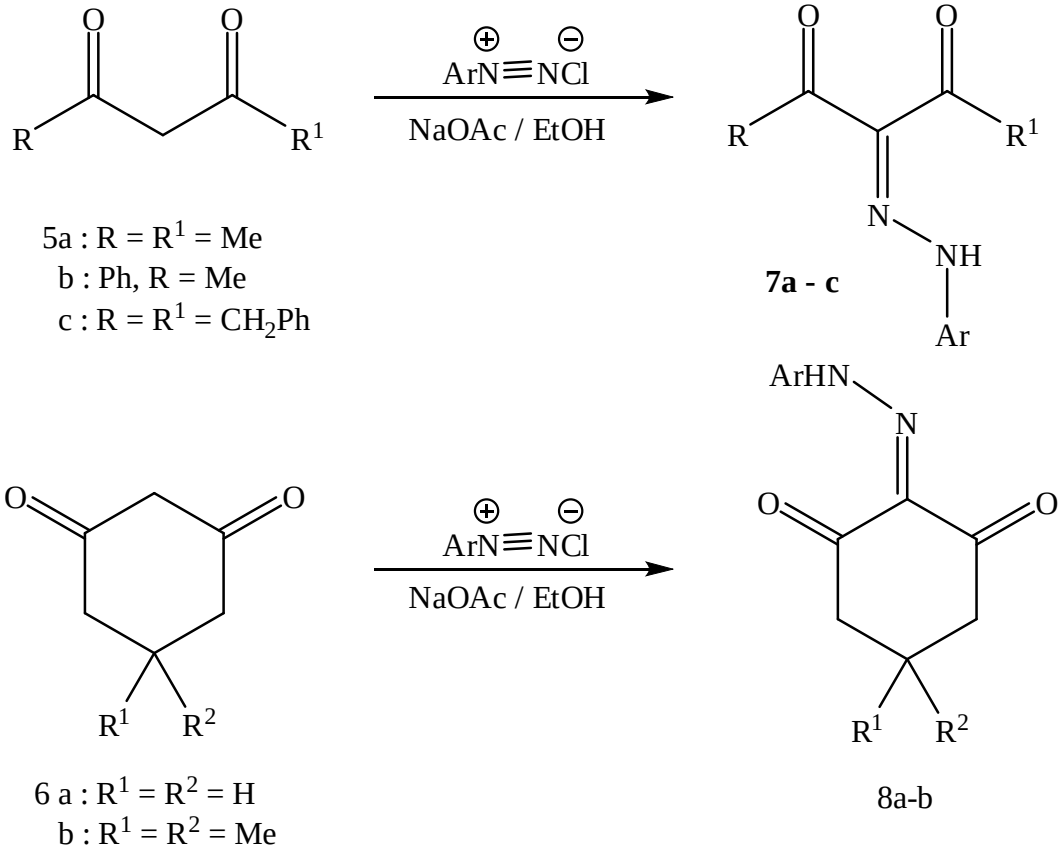
The required α–functional aldehyde and/or ketone can be easily provided by this route (Alexakis *et al.*, 1991). Condensation of glyoxal **1a** and substituted glyoxal **1b** and **1c** with substituted hydrazines leads to the formation of the corresponding mono or bis hydrazones **2** or **3** basing on the molar ratio and on the applied reaction conditions (Huisman *et al.*, 1997). It has been reported that **1b** reacts with phenylhydrazine to yield **4** which then rearranges into **2** (Scheme 1.2).



Scheme 1.2: Condensation of functionalized aldehyde and ketone with hydrazines

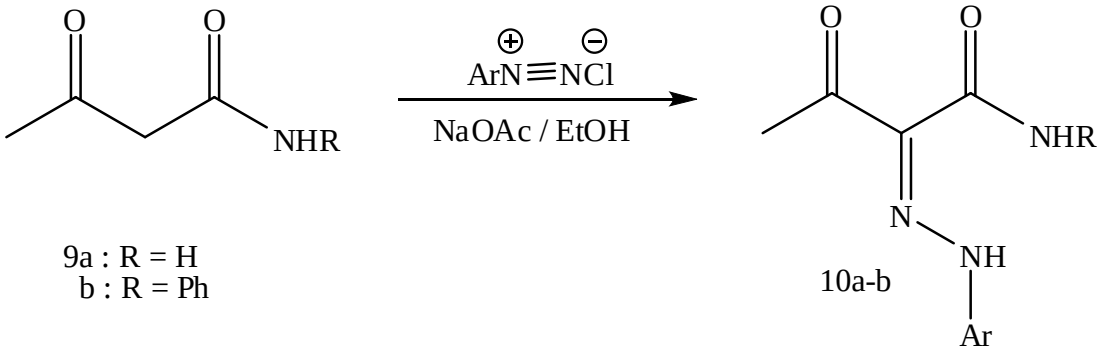
1.3.2 Coupling active methylenes with aromatic and heteroaromatic diazonium salts

Generally the coupling reaction is conducted at ambient temperature in protic solvents in the presence of a base. The most commonly used base is sodium acetate but reaction in the presence of sodium hydroxide or pyridine also has been reported (Al–Awadi *et al.,* 1995). In this step, acetylacetone **5a**, benzoylacetone **5b**, diphenylpentanedione **5c** and 1,3 - diketones (**6a, 6b**) have been coupled with aromatic diazonium salts in ethanolic sodium acetate solution yielding the corresponding products **7a - c** and **8a - b** (Al–Awadi *et al.,* 1995) (Scheme 1.3).



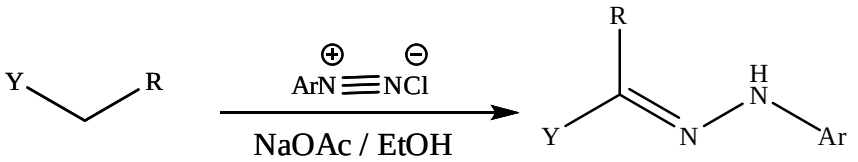
Scheme 1.3: Coupling active methylenes with aromatic and heteroaromatic diazonium salts

Similar coupling of oxobutanamide **9a** and oxophenylbutanamide can readily yield arylhydrazones **10a & b** (Sadanadam *et al.,* 1991) and were usefull as dyes and pigments (Scheme 1.4).



Scheme 1.4: Coupling of oxo- and oxophenylbutanamide with aromatic diazonium salts

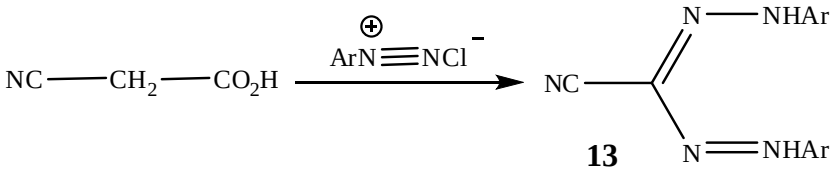
Ethyl acetoacetate **11a** (Kumar *et al.,* 2003), ethyl cyanoacetate **11b** (Brecknell *et al.,* 1969), cyanoanilides **11c** (Shawali and Abd El-Galil, 1971), have been reported to be readily coupled with aromatic diazonium salt in the presence of sodium acetate to yield the corresponding arylhydrazones **12a – c** but the exact geometry has only been established recently (Scheme 1.5).



11a: R = COMe; Y = CO_2Et
b: R = CN; Y = CO_2Et
c: R = CN; Y = CONHAr

Scheme 1.5: Coupling of acetato- and cyno-compounds with aromatic diazonium salts

The reactivity of methylene functions in acetonitrile and benzylcyanide towards aromatic diazonium salts is not sufficient. Attempted coupling of cyanoacetic acid with aromatic diazonium salt affords only formazans **13** (Cankař and Slouka, 2003) (Scheme 1.6).



Scheme 1.6: Coupling of cynoacetic acid with aromatic diazonium salts

1.4 Applications of hydrazones

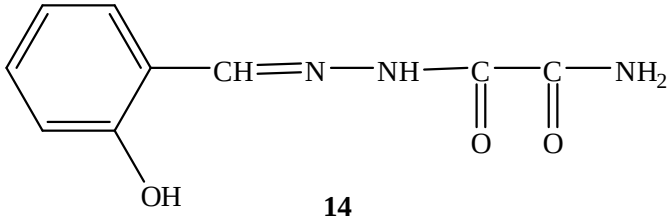
1.4.1 Analytical applications of hydrazones and their derivatives

Hydrazones have been intensively investigated and their chemical properties studied. In inorganic chemistry (mainly due to their facile synthesis), tuneable electronic and steric properties and good chelating ability (Carcelli *et al.*, 2005 and Das *et al.*, 2005) were reported. Derivatives of these compounds were investigated and used for the determination of glucose in biological fluids such as serum, plasma and urine (Gochman and Schmitz *et al.*, 1972). 3–methyl–2–benzothiazolone hydrazone was applied for the determination of glucose in serum or plasma as a blue cationic dye with glucose in aqueous solution was formed (Neeley and Cupas, 1973).

Hydrazone derivatives are widely used in various analytical applications (Zegota, 1999; Zlotorzynska and Lai, 1999; Cordis *et al.*, 1998; Lamberton *et al.*, 1974). They are also found to have versatile coordinating abilities towards different metal ions giving well-characterized metal complexes (Raj and Kurup, 2007). They act as multidentate ligands with metal ions (e.g., transition elements) forming coloured chelates which are then used in the selective and sensitive determination of metal ions (Vasilikiotis and Stratis, 1975). They are also used to determine airborne aldehydes and ketones in air samples by using 2,4–dinitrophenylhydrazine (DNPH) method in which the analytes were analyzed by passing a stream of air through DNPH coated silica gel test tubes which are immediately eluted with acetonitrile and injected into HPLC system (Vogel *et al.*, 2000). In addition they were used for the detection of formaldehyde in libraries (Hanoune *et al.*, 2006).

Fernando *et al*. (1986) synthesized N–oxalylamine (salicylaldehyde hydrazone), **14**. The Al^{3+}–**14** system exhibited fluorescence properties when tested in ethanol solution (λ_{ex} = 385 nm, λ_{em} = 470 nm) and in aqeous 4% v/v DMF (λ_{ex} = 370 nm, λ_{em} = 470 nm). The optimum concentration of the reagent was 3.86×10^{-4} M for 198 ppb of aluminum (Al). The

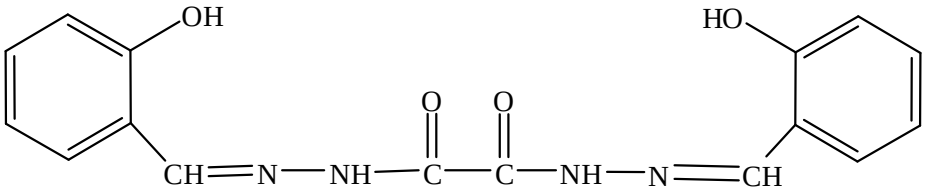
linear concentration range of Al was 0 – 160 ppb with relative error (P = 0.05) of ± 0.86%. This method was applied for the determination of Al in mineral waters.



14

Jose *et al*. (1984) synthesized N,N′-oxalylbis(salicylaldehyde hydrazone) **15**, which was used for the spectrophotometric and spectrofluorimetric determination of trace amounts of aluminium. The complex absorbs maximally at 390 nm and is linear over 0.1 – 1.8 g mL^{-1} Al. The molar absorptivity of the **15** – aluminium complex was 14.5 $\times 10^{3l}$ mol^{-1} cm^{-1}. Cations such as Hg^{2+}, Ni^{2+}, Pb^{2+}, In^{3+}, Ga^{3+} Ti^{4+}, V^{5+}, Cu^{2+}, Zr^{4+} and Fe^{3+} interfered. They were minimized by using thiosulphate as a masking agent and also by retention on a cation – exchange column.

The fluorimetric method for the determination of aluminium using **15** was linear over 0 - 200 ppb aluminium. Relative error is 6.7% (P = 0.05). Interferences of gallium, indium and thorium, also the effects of Pd^{2+}, Th^{4+}, Bi^{3+}, Ti^{4+}, V^{5+}, Cu^{2+}, Fe^{3+} and Ti^{4+} on the applicability of the reagent was also studied. The developed methods were applied for the determination of Al in alloys, pharmaceutical preparations and mineral water.

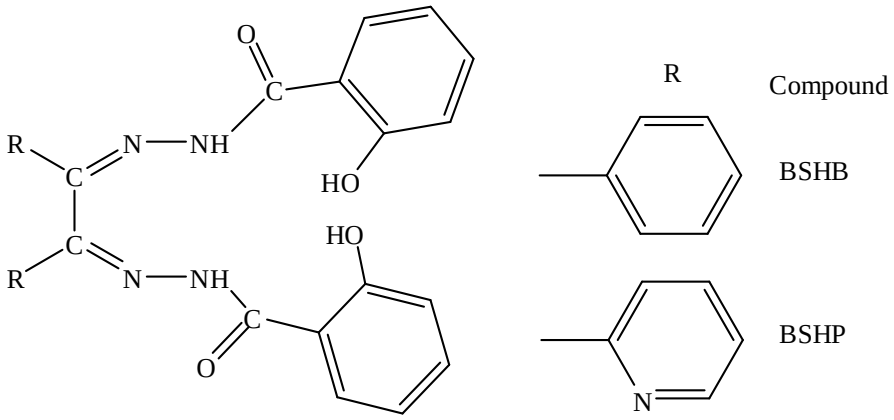


15

Silva and Valcàrcel, (1980) synthesized diphenylglyoxal bis(2– hydroxybonzoyl hydrazone) **16**, and was used for the spectrophotometric determination of calcium in water and foods. This compound reacts with calcium (II) at pH 12 to produce a yellow complex.

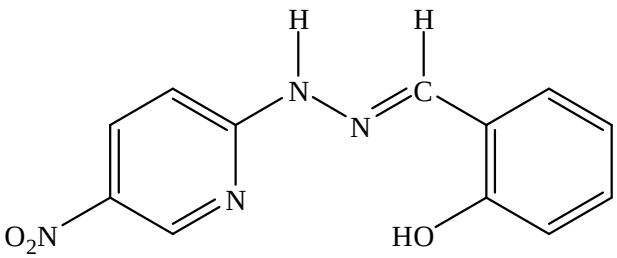
Beer's law was obeyed from 0.5 and 2.0 μg mL^{-1} and the molar absorptivity at 432 nm was $1.76 \pm 0.14 \times 10^4$ L mol^{-1} cm^{-1}.

Another reagent, **16** enables Beer's law to be obeyed from 0.75 – 3.0 ppm of calcium. Molar absorptivity was $1.03 \pm 0.05 \times 10^4$ L mol^{-1} cm^{-1}. The linear concentration range of calcium was from 1.24 – 2.50 ppm with minimum error (P = 0.05) as ± 32%. Interferences from strontium, barium and magnesium were also studied.



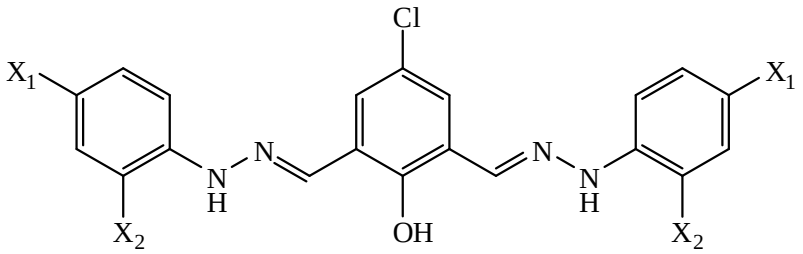
16

Chan and Cha, (1998) reported the synthesis of 2–hydroxybenzaldehyde–5–nitro–pyridylhydrazone **17**, and its application in the spectrophotometric determination of trace amounts of cobalt in the presence of surfactants after a column separation containing Amberlite resin. The method was linear over 0.02 – 2.0 mg L^{-1} and the limit of detection was 0.01 mg L^{-1}.

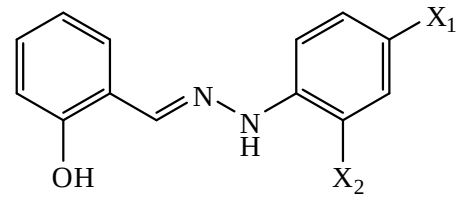


17

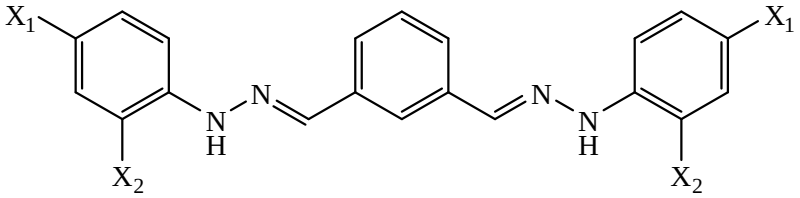
Zhang *et al*. (2009) synthesized a series of novel colorimetric anion sensors **18**(**a – f**) bearing phenol O–H and hydrazone N–H groups. Compounds **18a**, **b** and **d** showed selective recognition for acetate ion in DMSO/H_2O (7.5 : 2.5, v/v) solutions. Sensors **18** (**a** & **b)** could selectively sense chloride ion in DMSO/H_2O (9 : 1, v/v) binary solutions by the collaboration of phenol O–H and hydrazone N–H groups. Comparison with sensor **18e** indicates that the introduction of acidic phenol O–H group significantly enhanced the sensor′s affinity to the chloride ion, which also enhanced the sensitivity of the sensor for sensing acetate ion. It was found that the O–H group plays a critical role in the anion-recognition process



18a & b



18c & d



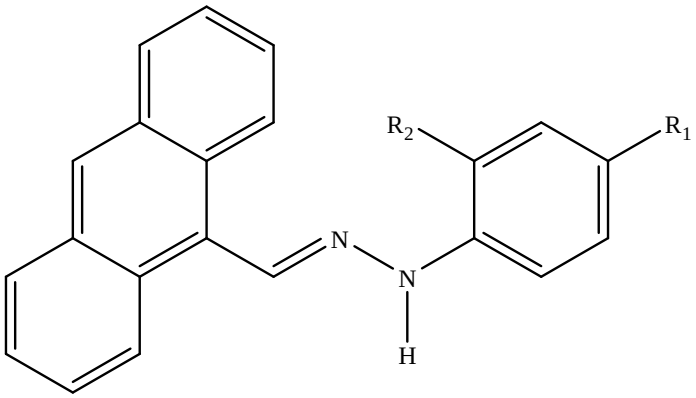
18e & f

.

18a, **c**, **e**: $X_1 = NO_2$, $X_2 = H$; **b**, **d**, **f**: $X_1 = X_2 = NO_2$

Shang and Xub, (2009) synthesized a series of artificial receptors and hydrazone derivatives containing anthracene (**19a-c)**. The reactivity of these compounds towards

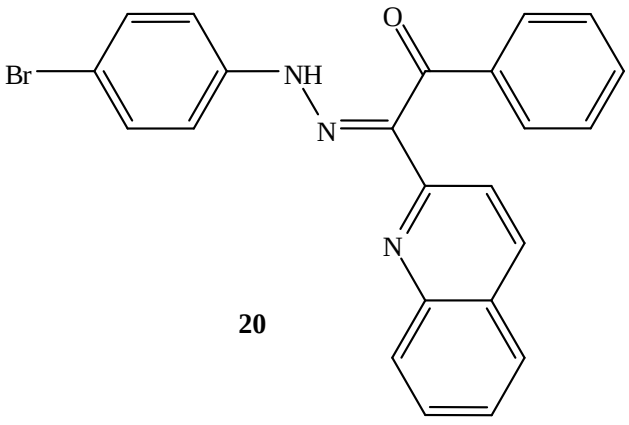
biologically important anions was studied by UV–vis, fluorescence and ^{1}H NMR titration experiments and theoretical investigations. Results showed that the reagent **19a** in the absence of NO_2 has no binding ability for various anions. While other reagents **19**(**b** & **c**) showed the highest binding ability towards various anions. Moreover, the colour changes took place during the binding between the host and guest, has made this system to be used as colorimetric anion sensor. The interaction of this reagent with the studied anions depends on the NO_2 which acts as a colorimetric and electron withdrawing group. The reagent is not affected by the presence of other anions.



a: $R_1 = R_2 = H$
b: $R_1 = NO_2$, $R_2 = H$
c: $R_1 = R_2 = NO_2$

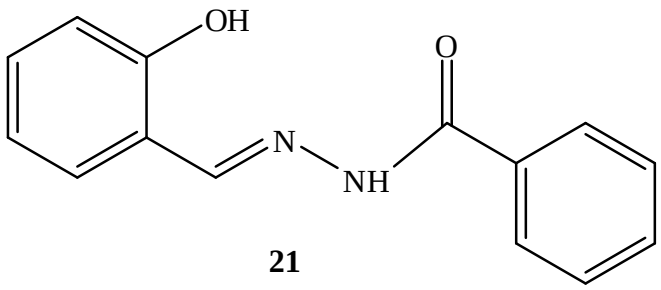
19

Hamid *et al*. (2005) prepared polyvinylchloride (PVC) – based membrane electrodes that are selective to lead ion, containing a neutral ionophore 1–phenyl–2-(2–quinolyl)–1,2–dioxo–2–(4–bromo)phenylhydrazone **20**. It was used as an indicator electrode for the potentiometric titration of lead ion with potassium dichromate.



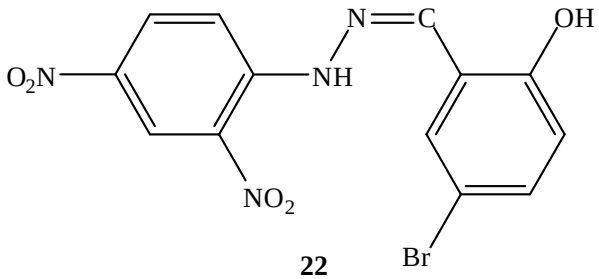
20

Ganjali *et al*. (2009) reported a highly selective and sensitive dysprosium (III) optical sensor based on N'-[(2-hydroxyphenyl)methylene] benzohydrazide (BBH) immbilized on triacetylcellulose **21**. The effect of various ions present in the sample was studied. This sensor was successfully applied for the direct determination of Dy (III) ions in urine and river water.



21

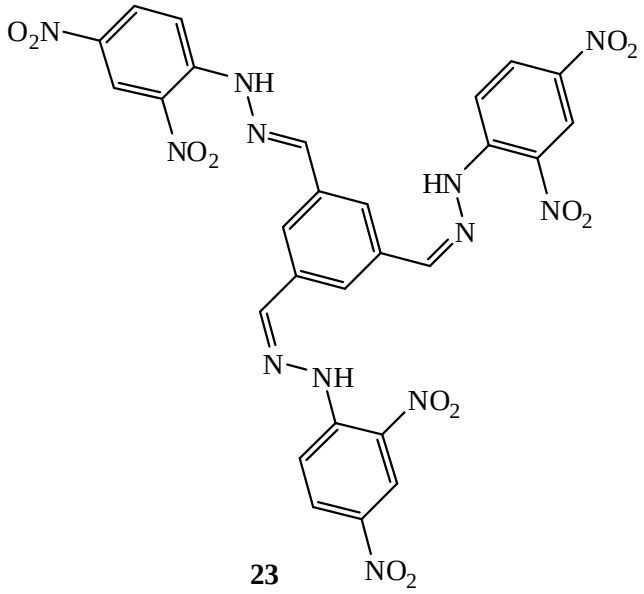
Jie Shao *et al*. (2008) investigated phenylhydrazone – based sensor **22** for the sensing of acetate in DMSO and in aqueous solutions. The change in colour of the reagent from yellow to red indicates its binding capability upon the addition of acetate. The intensity of the colour can be compared by the naked eye in which its colour induced by acetate is deep red while by phosphate or fluoride is light red. Acetate anion produced stronger complex than the other anions which allows acetate anion to be selectively detected from other anions.



22

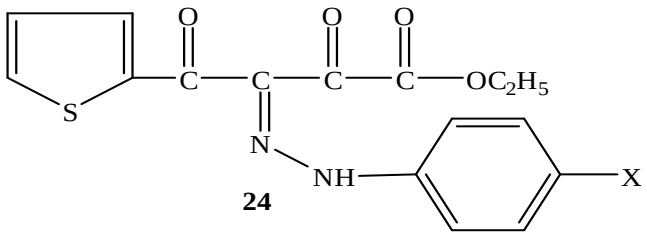
Jie Shao *et al*. (2009) reported that an anion receptor **23** based on a tripodal benzaldehydic – phenylhydrazone can selectively sense acetate ion by using UV – vis absorption spectroscopy in DMSO. The interaction of this reagent (host) with the acetate

anion (guest) was found to be in the ratio (1:3). The colour change disappears upon addition of water which disturbs the anion binding. The reagent has proven as an excellent sensor for anions such as fluoride and acetate in organic medium.



23

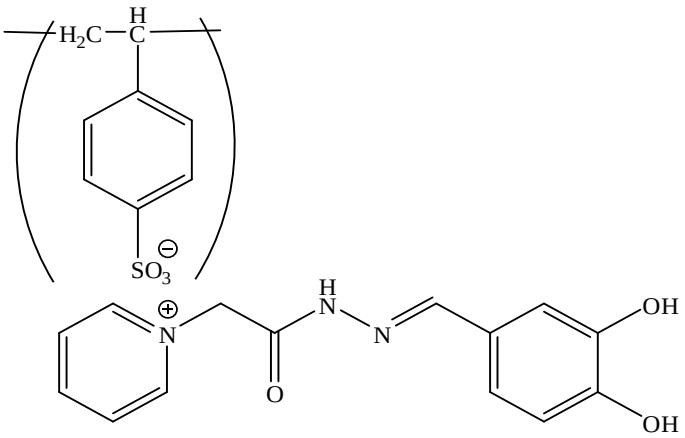
El–Seify and El-Dossoki, (2006) studied the electronic spectra of [3–(2–thenoyl) 3–(*p*–NO_2– phenylhydrazone) ethyl pyruvate] **24**, *p*–Br (II) and *p*–CH_3 in ethanol and the obtained spectra showed four absorption bands which are due to the corresponding electronic transition. Conductometric titrations have also been conducted and the results showed that this transition can be used for the determination of the metal ion and also the ligand concentrations. Polymeric membrane lanthanum (III)-selective electrode based on N,N'-adipylbis(5-plenylazosalicylaldehyde hydrazone) **24** was also reported (El-Seify and El-Dossoki, 2006).



24

(I): (X = NO_2; (II): (X = Br); (III): (CH_3)

Hassanien *et al.* (2008) has successfully separated chromium III & VI from different solutions using 1-(3,4–dihydroxybenzaldehyde)–2–acetylpyridinium chloride hydrazone **25** loaded on Duolite C20 in batch and column modes. The method is simple, fast and can easily be applied for the separation and determination of two cations simultaneously. The separation was done by using 100 mg modified resin with stirring time for 45 and 30 min. Cr(III) was separated at pH 6 and Cr(IV) at pH 2, respectively. The method showed high recovery (97–100%), satisfactory repeatability with RSD < 4% and it was applied successfully for the determination of chromium in tap water and synthetic mixtures by using the column technique.



25 DAPCH-Duolite C20

Hydrazones were used as potentiometric sensors for the determination of cation species. Some of these sensors are summarized in Table 1.1 and their structures shown in Figure 1.1.

Table 1.1 Some examples of potentiometric sensors based on hydrazone derivatives for the determination of cationic species.

Ligand no.*	Cation	Hydrazone ionophore	Linear range (M)	Slope (mV decade^{-1})	Interfering ions (log K_{sel} > -2)	Reference
26	Er^{3+}	*N′*-(2-hydroxy-1,2-diphenyl-ethyldiene)benzohydrazide	10^{-7}- 10^{-2}	21.0	-	Ganjali *et al*., 2006
27	Er^{3+}	Pyridine-2-carbaldehyde-2-(4methyl-1,3-benzothiazol-2-yl)hydrazone	10^{-5} - 10^{-2}	21.8	Sm^{3+}, Tm^{3+}, Gd^{3+}	Ganjali *et al*., 2007
28	Eu^{3+}	Bis(thiophenol)butane-2,3-dihydrazone	10^{-5} - 10^{-2}	19.8	La^{3+}, Gd^{3+} , Sm^{3+}	Ganjali *et al*., 2007
29	Hg^{2+}	Bis(2-hydroxy-benzophenone)- butane-2,3-dihydrazone	10^{-6} - 10^{-1}	29.7	Fe^{3+}, Cd^{2+}, La^{3+}	Ganjali *et al*., 2007
30	La^{3+}	Bis(2-methylbenzaldehyde)-butane-2,3-dihydrazone	10^{-5} - 10^{-1}	19.8	Cu^{2+}, Ce^{3+}, Pr^{3+}	Ganjali *et al*., 2005
31	La^{3+}	3-hydroxy- *N′* -(pyridin-2-ylmethylene)-2-naphthohydrazide	10^{-7} - 10^{-2}	19.2	Yb^{3+}, Ce^{3+}	Ganjali *et al*., 2006
32	La^{3+}	*N′* -(1-pyridine-2-y lmethylene)-2-furohydrazide	10^{-6} - 10^{-1}	19.2	Sm^{3+}, Nd^{3+}	Zamani *et al*., 2006
33	Ce^{3+}	*N'*-[(2-hydroxyphenyl)methylidene]-2-furohydrazide	10^{-5} - 10^{-1}	19.4	-	Zamani *et al*., 2007
34	Pr^{3+}	*N*-(pyridin-2-ylmethylene) benzohydrazide	10^{-2} - 10^{-6}	21.1	Sm^{3+}, Er^{3+}	Ganjali *et al*., 2006
35	Sm^{3+}	3-{[2-oxo-1(2H)-acenaphthylenyliden]amino}-2-thioxo-1,3-thiazolidin-4-one	10^{-6} - 10^{-1}	19.3	-	Zamani *et al*., 2007
36	Tm^{3+}	Thiophene-2-carbaldehyde-(7-methyl-1,3-benzothiazol-2-yl) hydrazone	10^{-5} - 10^{-2}	19.5	Er^{3+}	Ganjali *et al*., 2005
37	Yb^{3+}	3-hydroxy- *N′*-[(2-hydroxy-phenyl)-methylene]-2-naphthohydrazide	10^{-7} - 10^{-2}	19.2	Nd^{3+}, Pb^{2+}, Gd^{3+}	Ganjali *et al*., 2006

*= Please refer to Figure 1.1 for their structures.

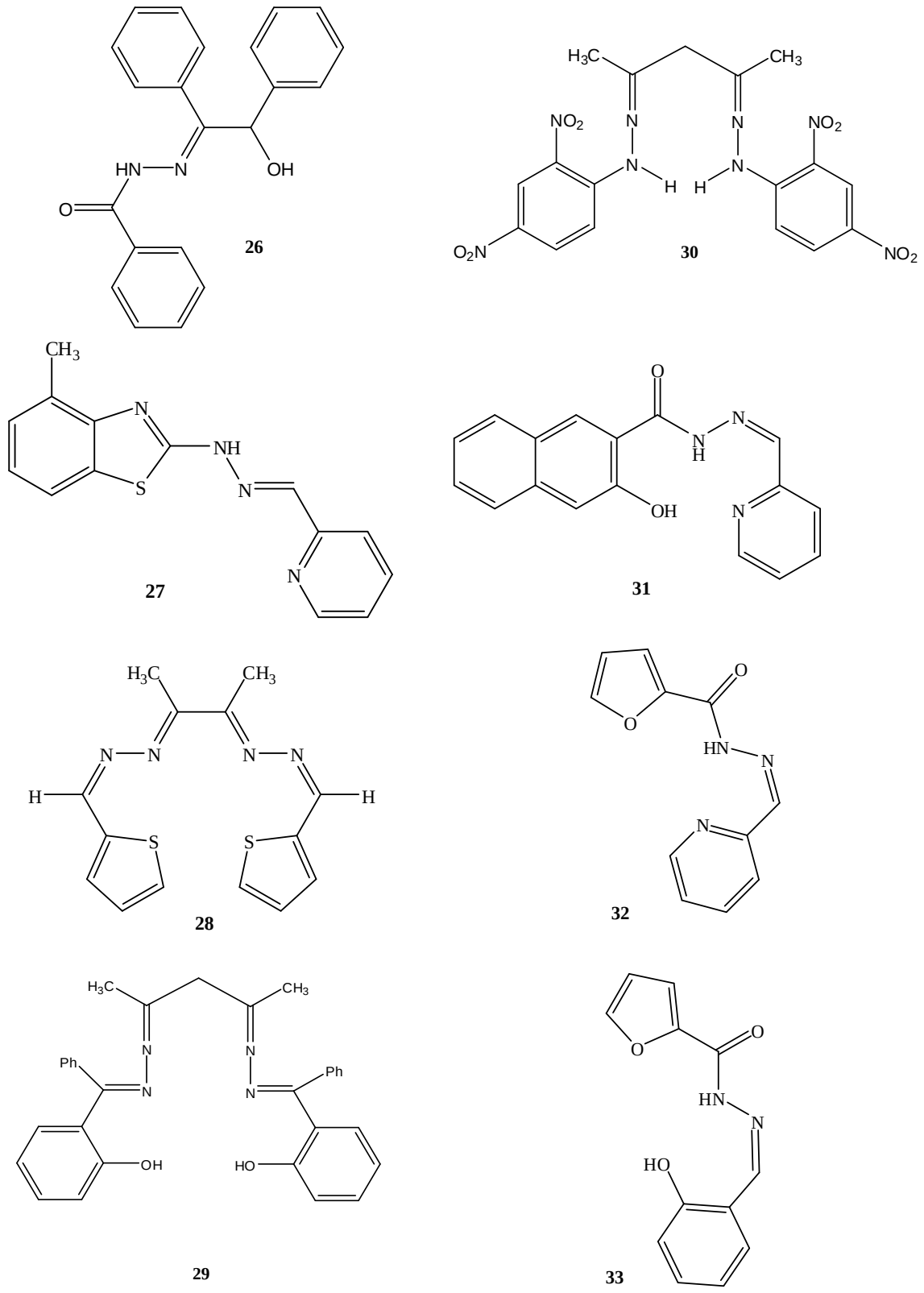


Figure 1.1 Structures of some hydrazone derivatives used as potentiometric sensors for the determination of cation species.

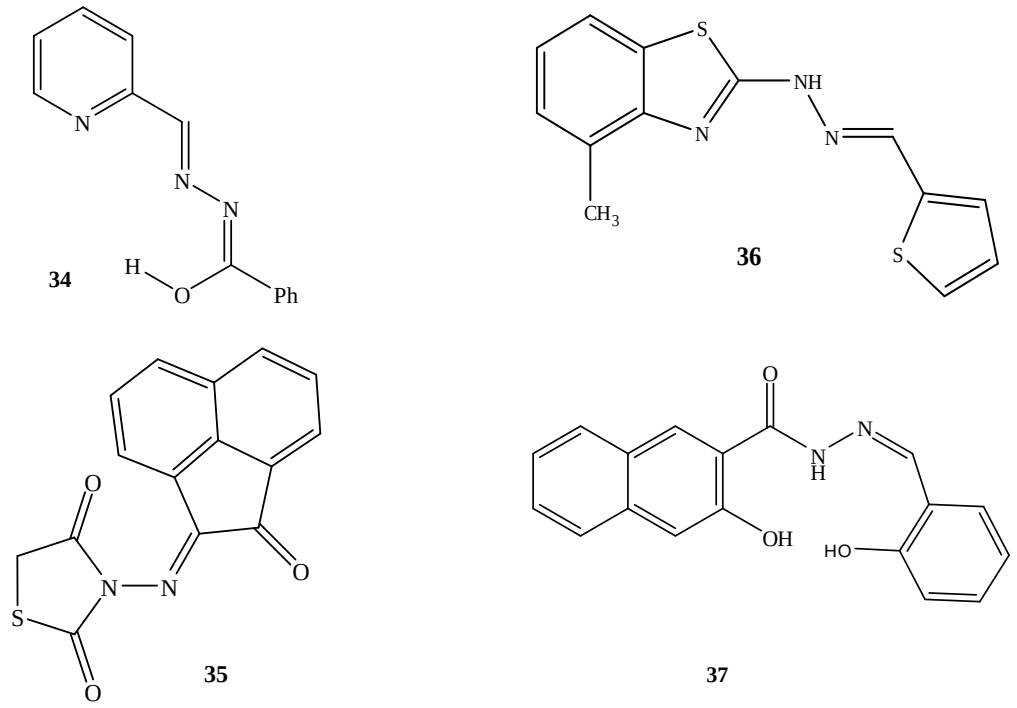


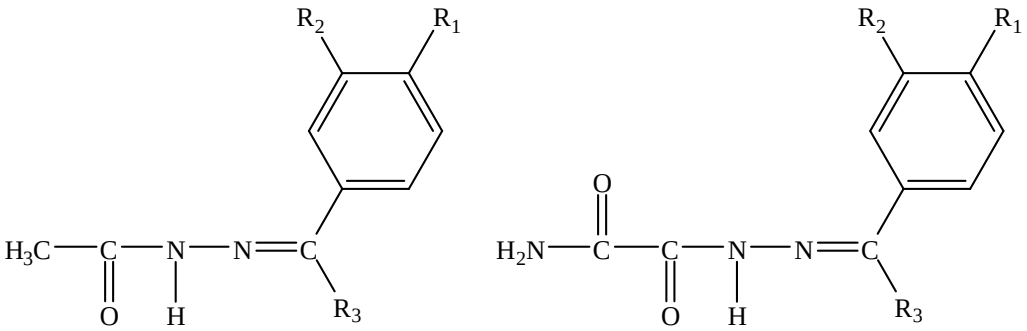
Figure 1.1. Continued.

1.4.2 Biological activity

Hydrazones have been extensively studied due to their biologically active properties such as anti-tumoral, analagesic, anti-platelet, anti-microbial, anti-tubercular and anti-inflammatory activities. For example, isonicotinoyl hydrazones have been reported to have anti-tubercular activity, also the activity of 4-hydroxybenzoic acid [(5-nitro-2-furyl) methylene]-hydrazide (nifuroxazide) was investigated and the results showed that this compound was active as an intestinal anti-septic. Moreover, Küçükgüzel *et al*. (1999) and Rollas *et al*. (2002) have found that 4-fluorobenzoic acid[(5-nitro-2-furyl)methylene]-hydrazide and 2,3,4-pentanetrione-3-[4-[[(5-nitro-2-furyl) methylene] hydrazino]carbonyl] phenyl]-hydrazone have anti-bacterial activity against both *Staphylococcus aureus* and *Mycobacterium* tuberculosis. In addition N^1-(4-methoxybenzamido)benzoyl]-N^2-[(5-nitro-2-furyl)methylene]hydrazine was prepared and tested for its anti-bacterial activity (Küçükgüzel *et al*., 2002). Recently the activities of some newly synthesized hydrazide hydrazones were investigated against *M. tuberculosis* (Kaymakçıoğlu *et al*., 2006).

1.4.2.1 Anti-convulsant activity

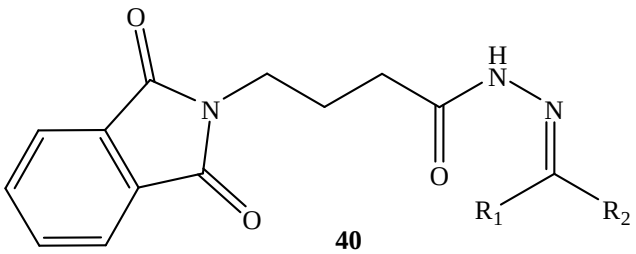
Biological tests indicated that, the activity of acetylhydrazone **38** was higher than that of oxamoylhydrazones **39** against convulsions. The results showed that the hydrogen bonding capabilities and anti-convulsant properties of these compounds was enhanced by the electron-donating groups (Dimmock *et al*., 2000).



38 **39**

R_1 = H, Cl, CH_3, OCH_3, Br;
R_2 = R_3 = H

A series of 4-aminobutyric acid (GABA) hydrazones **40** were synthesized and tested for their anti-convulsant properties in different animal models of epilepsy such as the maximal electroshock, the subcutaneous pentylenetetrazole, subcutaneous and intraperitonal picrotoxin induced seizure tests. Some of these derivatives were active in these models. Functionality of these compounds plays an important role in the anticonvulsant properties (Ragavendran *et al*., 2007).

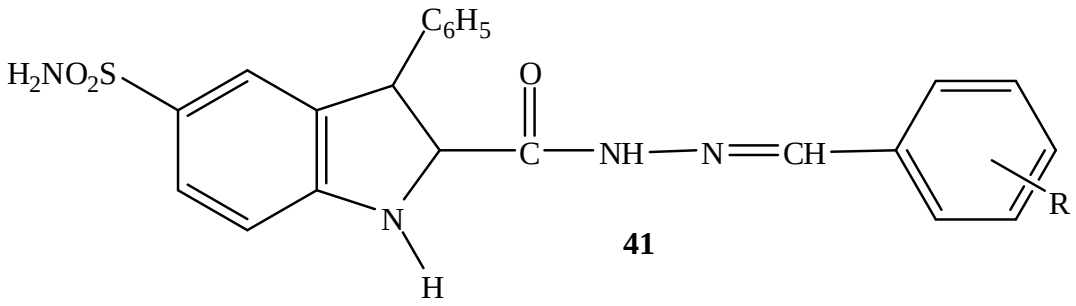


40

R_1 = H, 2-CH_3, 4-Br, 3-Cl ; R_2= 4-Cl, 2-CF, 3-F, 6-CH_3, 2-Br

1.4.2.2 Anti-depressant activity

A new group of compounds of arylidenehydrazides **41** which were resulted from the reaction of 3-phenyl-5-sulfonamidoindole-2-carboxylic acid hydrazide with different aldehydes and their anti-depressant activity was studied. All the prepared compounds showed anti-depressant activity at 100 mg kg^{-1} (Ergenç and Günay, 1998). Among this group, the most active compound was 3,4-methylenedioxy – substituted derivative.

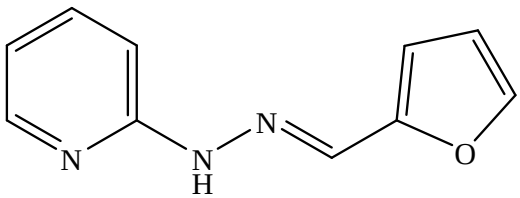


41

R= H, 4-Cl, 4-NO_2, 2-OCH_3, 4-N(CH_3), 4-CH_3, 2-OH, 4-F, 3,4-OCH_2O

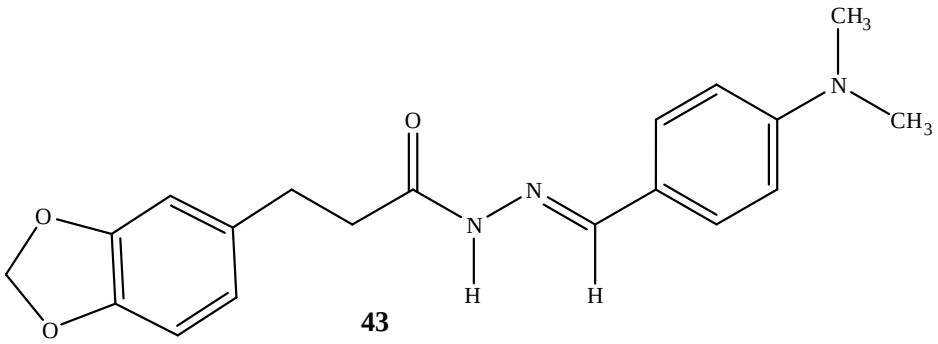
1.4.2.3 Anti-inflammatory activity

The biological activity of 2-(2-formylfuryl)pyridylhydrazone was studied and the results reported that this compound is useful as anti-inflammatory agent and gives a 79% inhibition of pleurisy at a dose of 80.1 µmol kg^{-1}. The optimum concentration for complexation of compound **42** with Ca^{2+} in vitro experiments was found to be 100 µM concentration (Todeschinia *et al*., 1998).



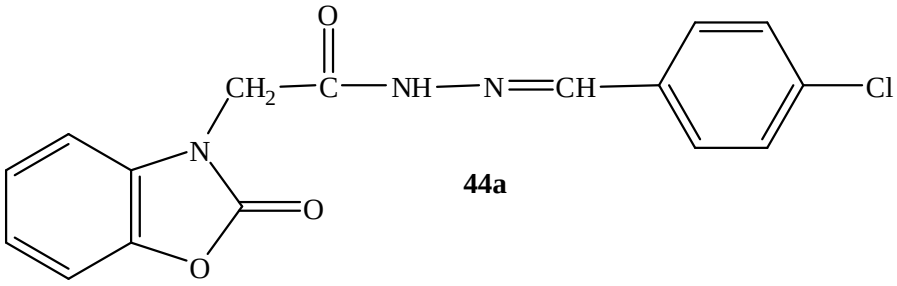
42

Lima and his co-workers, (2000) synthesized a new series of N-acylarylhydrazone anti-nociceptive compounds from natural safrole. **43** was more active than dipyrone and indomethacine, and are tested for their anti-inflammatory and anti-nociceptive activities.

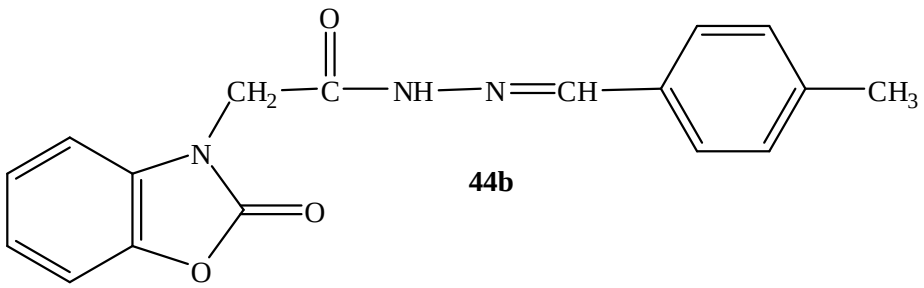


43

Salgin-Göşen *et al*. (2007) synthesized a series of 5-methyl-2-benzoxazolinone hydrazones. The analgesic-anti-inflammatory studies of 2-[2-(5-methyl-2-benzoxazoline- 3-yl)acetyl]-4-chloro-4-methyl benzylidene hydrazine **44a** and **44b** were conducted and the results showed that these compounds are highly active than those of morphine and aspirin. Moreover, 2-[2-(5-methyl-2-benzoxazoline-3-yl) acetyl]-4-methoxybenzylidene hydrazine exhibited the most anti-inflammatory activity at 200 mg kg^{-1} dose.



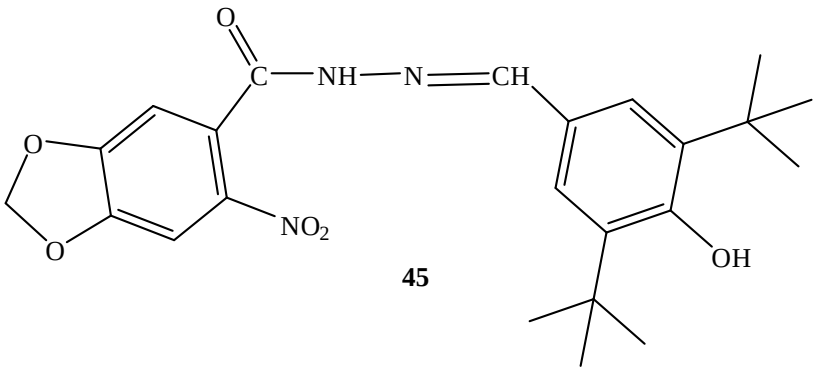
44a



44b

Duarte *et al*. (2007) described results on the testing of N\-(3,5-di-tert-butyl-4-hydroxybenzylidene)-6-nitro-1,3-benzodioxole-5-carbohydrazine **45** as a novel anti-

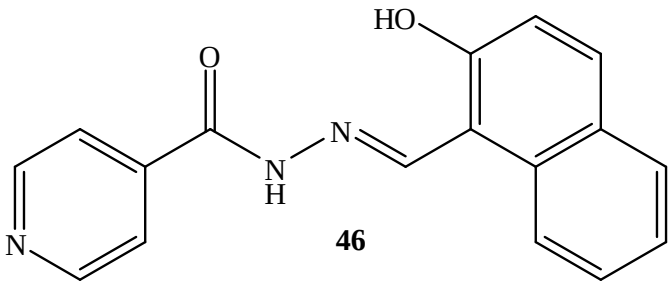
inflammatory compound. It was found that the activity of this compound depends on the 3,5-di-tert-butyl-4-hydroxyphenyl group attached to the imine function. This compound was tested by using 300 μmol kg^{-1} without any side effect of gastric irritation.



45

1.4.2.4 Anti-malarial activity

Due to the urgent need for more and highly effective anti-malarial drugs, the aroyhydrazone chelator 2-hydroxy-1-naphthylaldehyde isonicotinoyl hydrazone **46** have been studied and the results showed the high activity of this compound against malaria than desferrioxamine and chloroquine (Walcourt *et al*., 2004).



46

Bernardino *et al*. (2006) designed and synthesized a series of 1-substituted phenyl-N`- [(substituted phenyl) methylene]-1*H*-pyrazole-4-carbohydrazides **47** and examined their leishmanicidal and cytotoxic effects. In comparison with the ketoconazole, benznidazole, allopurinol and pentamidine drugs in vitro, the 1*H*-pyrazole-4-carbohydrazide derivatives with X = Br, Y = NO_2 and X = NO_2, Y = Cl showed the highest activity and more active against *L. amazonensis* than on *L. chagasi* and *L. braziliensis species*.